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THE GIANT EXTRA-FLORAL NECTARIES OF CARNIVOROUS *HELIAMPHORA FOLLICULATA*: ARCHITECTURE AND ULTRASTRUCTURE

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Extra-floral nectaries commonly occur in carnivorous plants, forming pitfall traps to attract nectar-feeding insects. Although they are not connected with pollination, extra-floral nectaries promote the reproductive functions of carnivorous plants by increasing the supply of animal-sourced nutrients and thereby increasing the plant's vigor. Our main purpose here was to study the functional ultrastructure of the giant nectaries in *Heliamphora*, focusing on nectar production and secretion. We wanted to determine whether specialization of the shape and structure of *Heliamphora* nectar spoons has an influence on nectary structure. *Heliamphora folliculata*, with its unique nectar storage chamber, may also have specialized giant nectaries differing from other species in the genus. In *Heliamphora folliculata* the largest nectaries occur in a nectar storage chamber. Regardless of their size, the nectaries have similar ultrastructure. Key features of their cells are ER-sheathed leucoplasts and vacuoles with large osmiophilic phenolic inclusions. The former is characteristic for cells producing monoterpenes; indeed, the giant nectaries produce volatile compounds and may have a function similar to osmophores. Nectary cells are isolated from ordinary parenchyma cells by cutinized walls lacking plasmodesmata (endodermis). Symplastic transport is possible only between nectary cells and special parenchyma cells that have wall thickenings. Between them are many plasmodesmata; thus the nectary is a symplastic and apoplastic field. These specialized parenchyma cells are similar to the flange cells described in parasitic plants. Why has a special spoon with a nectar chamber evolved in *Heliamphora folliculata*? One answer given is that it protects nectar against being washed away by frequent rainfalls so that the plant produces less nectar and saves energy. Also, when nectar is not easily accessible the insects have to spend more time near the trap entrance to look for it, and they are more likely to be trapped. Regardless of the shape and structure of *Heliamphora* nectar spoons (pitcher appendages), giant nectaries apparently have the same architecture throughout the genus. So far as is known, pollinator-prey conflict does not exist in *Heliamphora*; nectaries in this genus are formed only for nectar-feeding prey.

Key words: *Heliamphora*, Sarraceniaceae, extra-floral nectaries, nectar, carnivorous plants, ultrastructure, symplastic field, osmophores, leucoplasts, carnivorous syndrome, tepui.

INTRODUCTION

The family Sarraceniaceae, with three genera (*Darlingtonia, Heliamphora, Sarracenia*), groups carnivorous plants from the New World forming mainly pitfall traps (excepting *Sarracenia psittacina* with a lobster-type trap; Studnička, 1984). According to recent molecular studies, the genus *Sarracenia* is sister to *Heliamphora*, and this pair is sister to the genus *Darlingtonia* (Bayer et al., 1996; Neyland and Merchant, 2006). The genus *Heliamphora* comprises about 13 species and is restricted to tepuis and plateaus of Venezuela, Brazil and Guyana (Rice, 2006). Like other pitcher plants (*Cephalotus, Nepenthes, Darlingtonia, Sarracenia*), *Heliamphora* possesses extra-floral nectaries on the pitcher trap surface. Nectar-feeding insects are attracted and trapped (Jaffe et al., 1995). Their carcasses are a source of nutrients for the plants (Lloyd, 1942; Juniper et al., 1989; Jaffe et al., 1992). The anatomy of nectaries of a few species of *Heliamphora* were first described by Kraft (1896, after Lloyd, 1942) and later in detail by Lloyd (1942). Nectary morphology has been studied by SEM in only two *Heliamphora* species (*H. heterodoxa, H. nutans*), by Adams and Smith (1977) and Juniper et al. (1989); they report-

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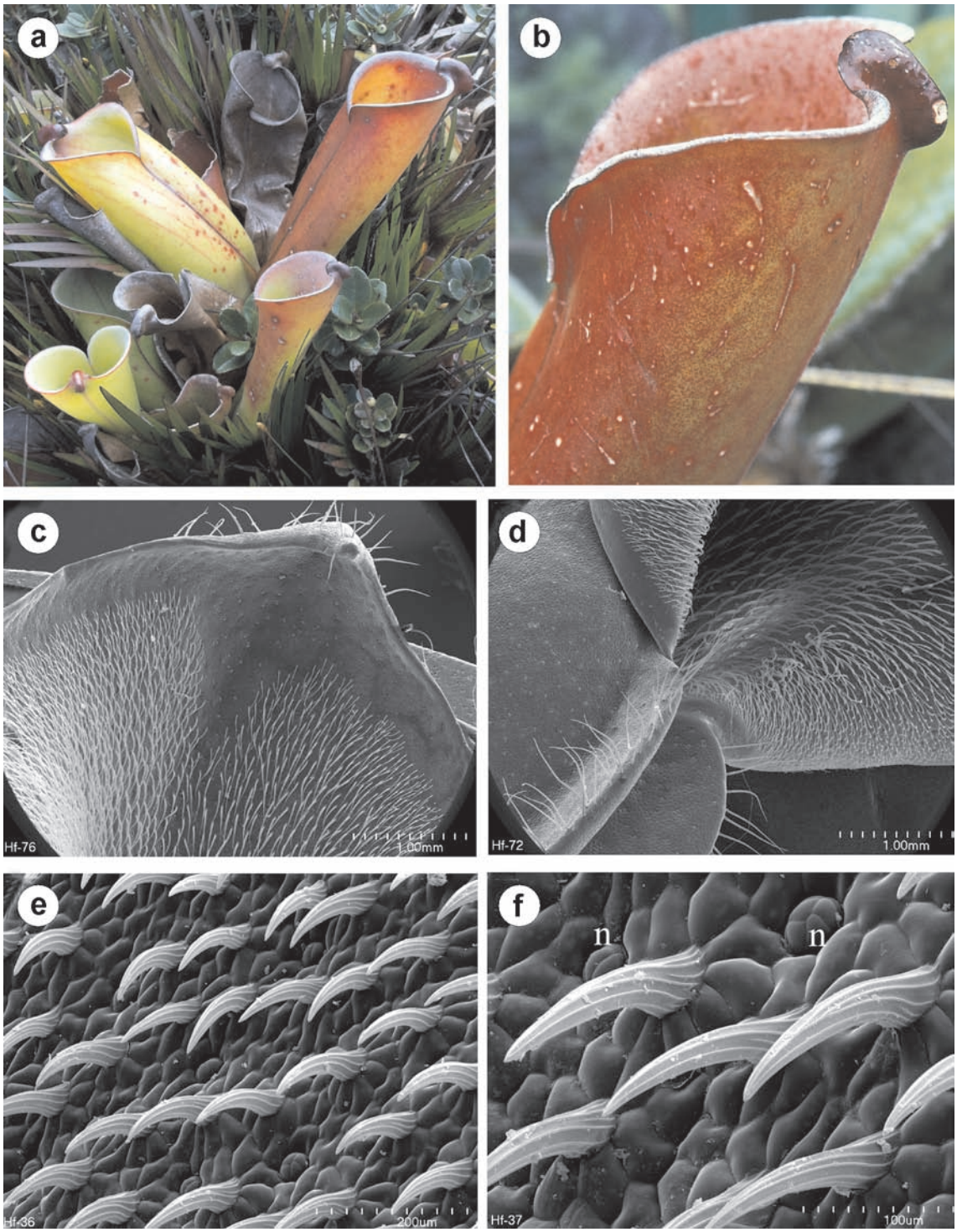

Fig. 1. (**a**) *Heliamphora folliculata* in its natural habitat in southern Venezuela, (**b**) Upper part of pitcher with nectar spoon (nectar chamber damaged), southern Venezuela, (**c**) Upper part of pitcher of juvenile plant, lacking the spoon; note glabrous triangular part with numerous nectaries, (**d**) Part of leaf of juvenile plant; on the external wings are long, straight, nonsecretory hairs together with numerous glands, (**e,f**) Small nectaries among downward-directed, unicellular, nonsecretory epidermal hairs, in adult plant. n – nectary.

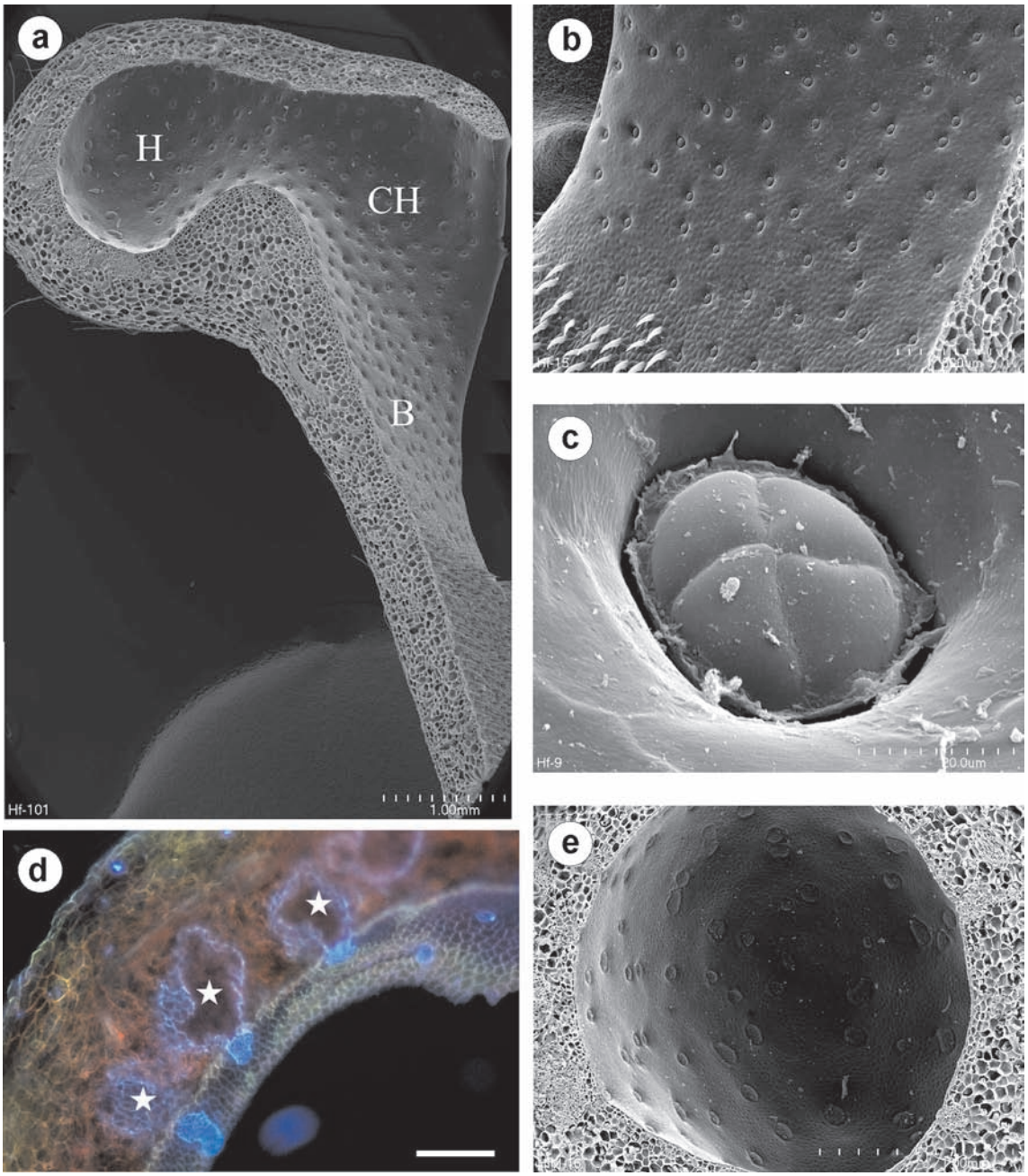

Fig. 2. (**a**) Section through nectar spoon. H – nectar chamber; CH – nectar channel; B – base, (**b**) Numerous small nectaries on glabrous spoon base, (**c**) Small nectary with four apical cells and remnants of secretion, (**d**) Part of section through nectar chamber; giant nectaries visible (*). Note strong fluorescence of cutinized walls. Bar = 300 μm, (**e**) Section through nectar chamber; numerous giant nectaries visible.

ed two kinds of nectaries in *Heliamphora*: large ones restricted to the pitcher appendage (spoon), and small ones occurring on the outer and partly on the inner pitcher surface. The small nectaries have a basic construction very similar to that of the extra-floral nectaries of *Sarracenia*, "*Sarracenia*-type" (Lloyd, 1942; Vogel, 1998). The giant extra-floral nectaries in Sarraceniaceae are restricted to *Heliamphora*. There have been no ultrastructure studies of nectaries in this genus. Vogel (1998) pointed to the need for work on the detailed ultrastructure of the nectaries of other members of Sarraceniaceae. To date, the only group of carnivorous plants to be studied in detail in terms of nectary ultrastructure and nectar production is

Nepenthes (Vassilyev, 1977; Juniper et al, 1989; Merbach et al., 2001).

Heliamphora folliculata Wistuba, Harbarth & Carow was described only recently, in 2001, from the Los Testigos table mountains in southern Venezuela. This endemic species is unique not only among *Heliamphora* species but also in the Sarraceniaceae family, in that its pitcher appendage (spoon) forms a chamber to store nectar (Fig. 1a,b; Wistuba et al., 2001). The structure and shape of pitcher appendages with nectaries is one of the most valuable characters for interpreting *Heliamphora* relationships (Wistuba et al., 2001, 2002 and Carow et al., 2005).

One of our aims here is to study nectar chamber anatomy and the distribution of nectaries in *Heliamphora folliculata*. The main purpose is to examine the ultrastructure of the giant nectaries of *Heliamphora*, focusing on nectar secretion. Another important aim was to determine whether specialization of the shape and structure of *Heliamphora* nectar spoons has an influence on nectary structure. Perhaps *Heliamphora folliculata*, with its unique nectar storage chamber, also has specialized giant nectaries differing from other species in the genus.

MATERIALS AND METHODS

PLANT MATERIAL

Juvenile and adult plants of *Heliamphora folliculata*, originated from seeds collected by Andreas Wistuba during a field trip in 2001 (Wistuba et al., 2001) from Aparaman Tepui and Murosipan Tepui, were examined. Additionally, *Heliamphora folliculata* plants from the Murosipan Tepui and plants of *Heliamphora minor* (Auyan Tepui) and *Heliamphora heterodoxa* were obtained from the collection of Kamil Pásek of Dobroslavice, Czech Republic.

LIGHT AND TRANSMISSION ELECTRON MICROSCOPY

The procedures for preparing samples for TEM were as described earlier (Płachno et al., 2007). Briefly, spoons with nectaries were hand-sectioned with a razor blade and fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0) for 4 h at room temperature. The material was post-fixed in 1% OsO_4 in cacodylate buffer for 24 h at 4°C, rinsed in the same buffer, treated with 1% uranyl acetate in distilled water for 1 h, dehydrated with acetone and embedded in Epon 812 (Fullam, Latham, NY). Semithin sections were stained with methylene blue and examined with an Olympus BX60 microscope. Ultrathin sections were cut on a Leica Ultracut UCT ultramicrotome. After contrasting with uranyl acetate and lead citrate, the sections were examined in a Hitachi H500 electron microscope.

SCANNING ELECTRON MICROSCOPY

The procedures for preparing samples for conventional SEM were as described earlier (Płachno et al., 2005a,b). Briefly, traps were hand-sectioned with a razor blade and fixed as for TEM or in 70% ethanol with 1% glycerine. The material was later dehydrated in an ethanol and acetone series, and critical-point dried using liquid CO_2. The dried tissues were sputter-coated with gold and viewed in a HITACHI S–4700 microscope (Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University). To study pit distribution and cell wall architecture, we extracted cytoplasm. Nectar spoons were hand-sectioned and fresh tissue was placed in commercial bleach (sodium hypochlorite) diluted 1:10 in water. After 2 h the material was washed in water and fixed in 2.5% glutaraldehyde in cacodylate buffer. Later procedures for preparing samples for SEM were as above.

HISTOCHEMISTRY

Autofluorescence (chlorophyll a, cutinized walls) observations of fresh sectioned tissue were made in an epifluorescence microscope (Nikon Eclipse E 400 with UV–2A and B–2A filters). For some samples DAPI was added to label DNA. Documentation was made on Sensia 200 and 100 slide film.

RESULTS

SPOON ANATOMY AND NECTARY DISTRIBUTION

Leaves of juvenile plants lack the spoon; only the apex of the pitcher is slightly curved and forms a small appendix at the tip (Fig. 1c). There are only small nectaries scattered on the glabrous inner surface of the apex, the glabrous triangular part of the upper inner pitcher wall, and the glabrous pitcher margins. As in mature traps, nectaries occur also on the inner pitcher wall among downward-directed, unicellular, nonsecretory epidermal hairs (Fig. 1d,e,f). Glands similar to small nectaries occur on the external pitcher surface and also on the wing surface. Long, straight, nonsecretory hairs occur on the external apical surface and wings of the pitcher (Fig. 1d).

In adult plants the spoon consists of three primary parts: the base, nectar channel and nectar chamber (Fig. 2a). Its massive construction comprises several layers of large parenchyma cells, vascular tissue and the epidermis. The parenchyma cells are characterized by the presence of a large vacuole and a thin layer of cytoplasm with small chloroplasts having a developed thylakoid system and small starch grains. In general the vacuolar content is electron-translucent, with osmiophilic inclusions. The epidermis is composed of a single layer of

flat, pavement-like, elongated cells, which are highly vacuolated. The vacuoles have electron-translucent content or contain electron-dense material (anthocyanin pigments). There are simple pits between neighboring epidermal cells. On the external spoon surface are V-shaped nonsecretory hairs. Nectaries occur on both the outer and inner surface of the spoon, but are especially densely distributed on the inner surface (Fig. 2b). The most common ones are small nectaries with a few apical cells, 3 or 4 in number, each of which is recessed in a pit (Fig. 2c). Regardless of nectary size, they all are almost completely sunken in the spoon tissue (Fig. 2d). The largest nectaries are almost all restricted to the nectar reservoir chamber (Fig. 2d,e), but some are found on the nectar channel surface. Several collateral vascular bundles supply the spoon's nectar reservoir (Fig. 3a); some bundles are near giant nectaries (Fig. 3b).

ARCHITECTURE OF GIANT NECTARIES

Nectary architecture of *Heliamphora folliculata* resembles that of *Heliamphora minor* and *Heliamphora heterodoxa*. The basic structure of the nectaries is uniform throughout the genus. The largest nectaries of *Heliamphora folliculata* are ~430 μm wide and ~450 μm long. Their true size is difficult to measure because the nectary tissue is lobed (Fig. 3b). They are almost completely sunken in the parenchyma; only a small part of the nectary – the apical cells – have contact with the chamber environment (Fig. 3c). There are up to ~50 apical cells, which form a dome or a more kidney-shaped structure (Fig. 3c). They have thick cutinized walls well visible by fluorescence microscopy (Fig. 3d) and in semithin sections after staining with methylene blue. Some subapical cells of the nectary have cutinized walls between other nectary cells. Nectary cells are isolated from ordinary parenchyma cells by cutinized walls (Fig. 3e) lacking plasmodesmata. Note that these peripheral nectary cells also have a partially cutinized radial wall (Fig. 3e)

Some peripheral nectary cells have contact with special parenchyma cells which have secondary wall thickenings in the form of flanges (flange cells) (Fig. 4a). Secondary wall thickenings occur at the side where the cell has contact with the nectary. They have a reticulate pattern similar to the tertiary wall thickenings of the tracheary element (Fig. 4b,c). The thickenings are especially prominent in flange cells of small nectaries. In sections the thickenings are more or less dome-shaped and lignified, with an irregular surface (Fig. 4d). Between the thickenings, the primary wall is perforated by numerous plasmodesmata (Fig. 4c). The primary wall is partially cutinized (Fig. 4d). There are simple pits between the flange cells and parenchyma cells (Fig. 4e). The flange cells are highly vacuolated, with a prominent nucleus and small plastids containing tiny starch grains, and abundant mitochondria near the plasmodesmata (Fig. 4d).

NECTARY CELL ULTRASTRUCTURE

The nectary cells have dense cytoplasm with abundant organelles (Fig. 5a). Mitochondria are numerous and exhibit well-developed cristae; some are in close contact with the plasma membrane. The plastidome is well developed and consists of numerous leucoplasts, which lack starch grains but have darkly staining stroma, an electron-lucent network of internal membranes, and electron-lucent plastoglobuli (Fig. 5b). Some leucoplasts are pleomorphic and/or multilobed (Fig. 5c), or are cup-shaped and envelop the cytoplasm. ER surrounds the leucoplasts and forms long cisternae (Fig. 5 b). Cortical ER elements are in close contact with the plasma membrane (Fig. 5d). Dictyosomes are frequent and may occur in small groups (Fig. 6a). Each one consists of 7 or 8 cisternae; the dictyosomes seem to be active and associated with large vesicles (Fig. 6a). Vesicles with osmiophilic deposits fuse with the tonoplast and release the deposits to the vacuolar sap. Similar deposits also occur in the cytoplasm (Fig. 6b-d). Each cell has a large vacuole containing giant, spherical, osmiophilic inclusions with a wrinkled appearance (Fig. 7a). In the upper cells of giant nectaries, inclusions fill the whole vacuole (Fig. 7b,c). In these cells, flocculent material appears mainly on the peripheries of inclusions (Fig. 7b). In smaller nectaries this material occurs not only in upper but also in other nectary cells. Small electron-dense spherical deposits occur on the inner tonoplast surface. Small lipid droplets are also present in the cytoplasm (Fig. 6d). Multivesicular bodies may occur near dictyosomes (Fig. 6c). There are often paramural bodies containing vesicles of various sizes (Fig. 6d). The cell walls are thin and have a slightly undulating surface. In some cells, grey material occurs in the periplasmic space (Fig. 7d). There are intercellular spaces between nectary cells, some filled with grey material (Fig. 6c). Plasmodesmata were infrequently observed between nectary cells.

APICAL NECTARY CELLS

Apical cells have an ultrastructure similar to that described above for nectary cells, except for their very thick outer wall (Fig. 8a,b). Between the outer cell wall and plasmalemma is a thick layer of amorphous grey material with a delicate electron-dense network (Fig. 8b,c). The cuticle proper is developed as a thin, intact, electron-dense layer (Fig. 8b). Interestingly, the lateral apical cell of the nectary has a two-layered outer cell wall (Figs. 3d,e; 8d,e). The outer layer is strongly cutinized and continuous with the cutinized walls of peripheral nectary cells (Fig. 8d,e).

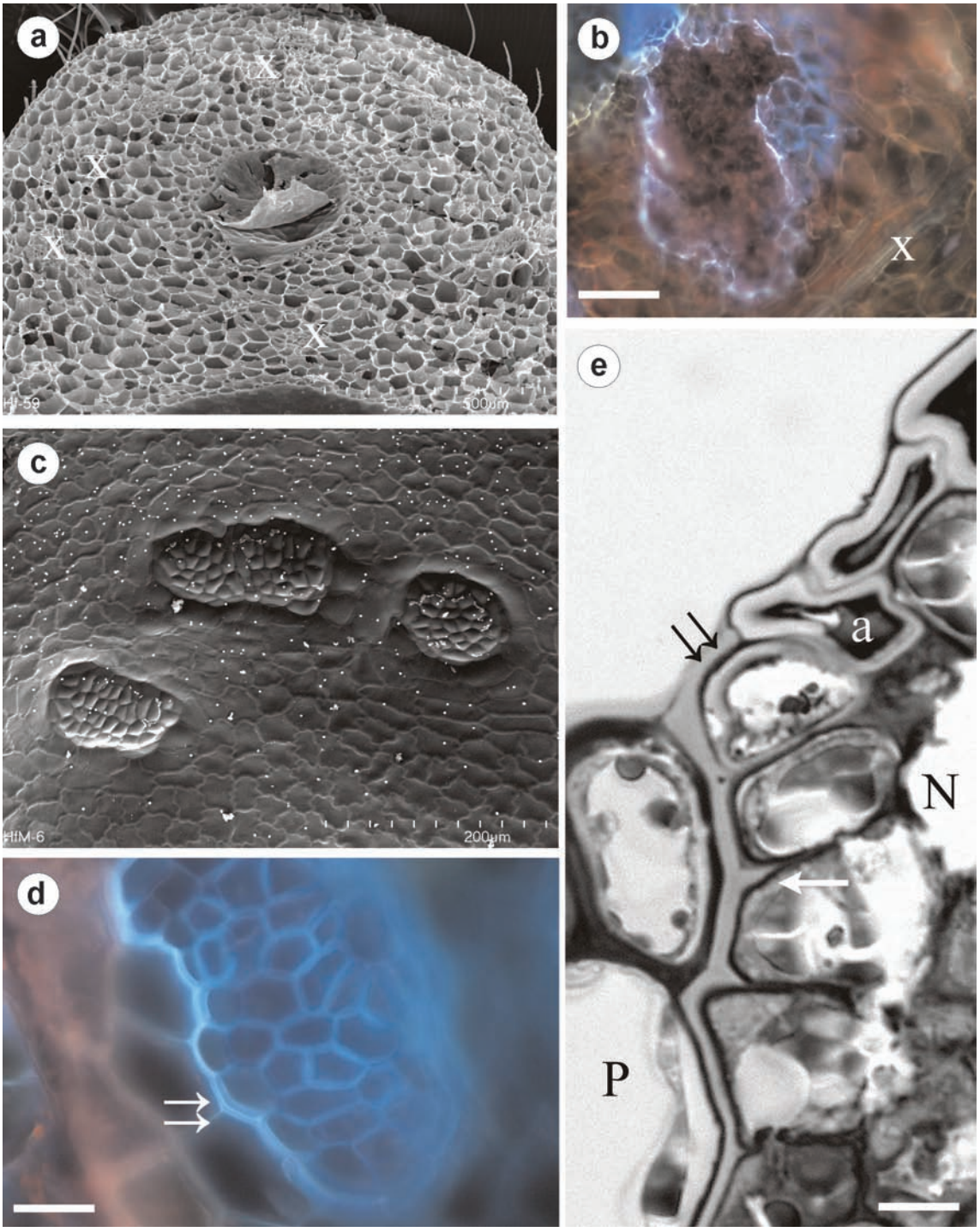

Fig. 3. (**a**) Part of section through upper part of nectar spoon, showing numerous vascular bundles (x) and part of nectar chamber with nectar remnants, (**b**) Section through giant nectary from nectar chamber. x – vascular bundle. Bar = ~125 µm, (**c**) Giant nectaries from nectar chamber, (**d**) Apical cells of giant nectary; note two-layered outer cell wall of lateral apical cell (double arrows). Bar = ~ 23 µm, (**e**) Part of semithin section through giant nectary. N – nectary; a – apical cell; double arrows – two-layered outer cell wall of lateral apical cell; arrow – cutinized radial wall of peripheral nectary cell; P – spoon parenchyma. Bar = ~ 5,3 µm.

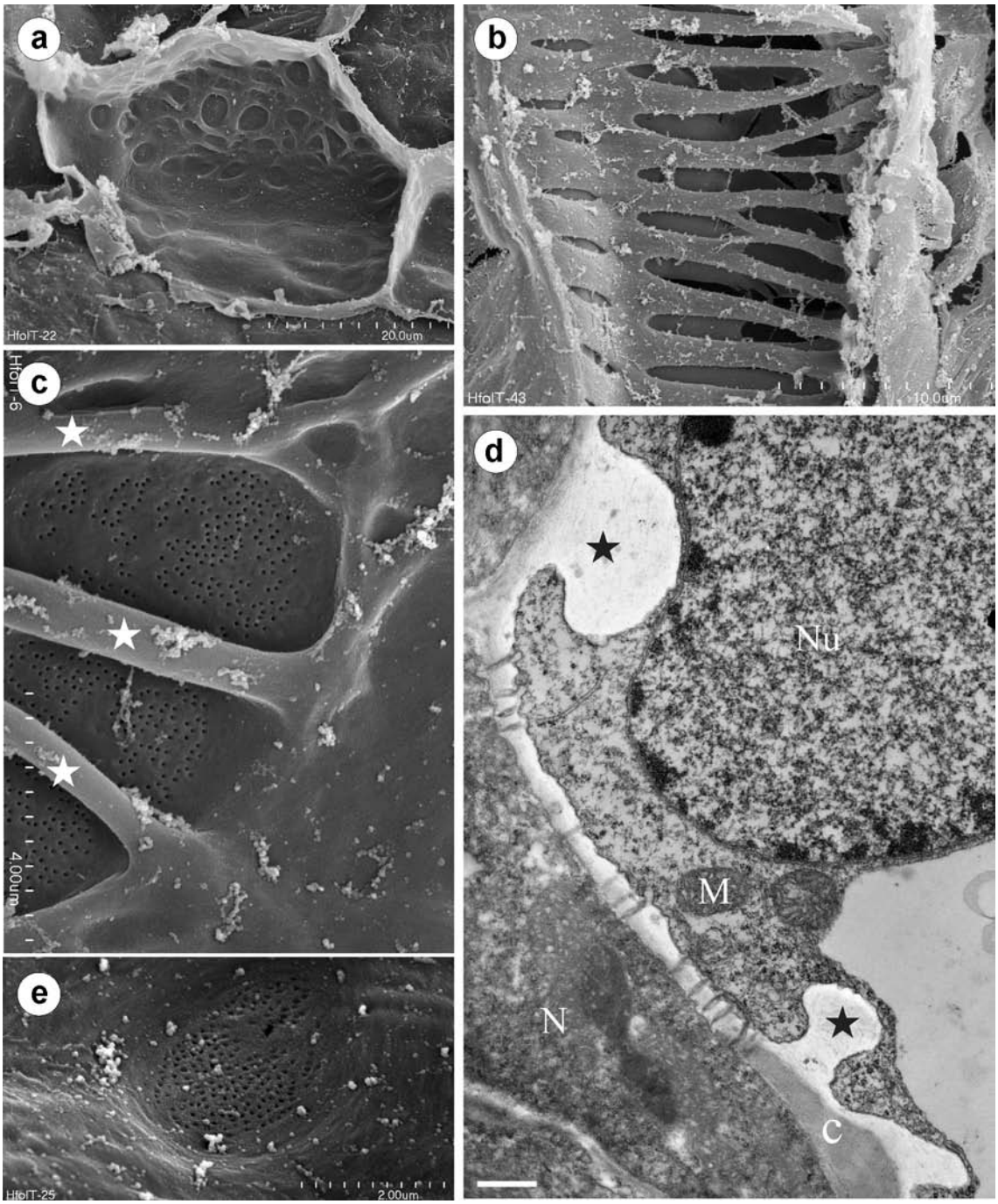

Fig. 4. (**a**) Flange cells; wall thickenings occur at the side in contact with the nectary, (**b**) Tertiary wall thickenings of tracheary element, (**c**) Part of section through flange cell; secondary wall thickenings (*) and numerous plasmodesmata are well visible after digestion of cytoplasm, (**d**) Part of section through flange cell and nectary cells; note numerous plasmodesmata. * – secondary wall thickening; c – cutinized cell wall; M – mitochondrion; Nu – nucleus; N – nectary cell. Bar = 0.44 μm. (**e**) Simple pit between flange cell and parenchyma cell.

DISCUSSION

Generally the nectary architecture of *Heliamphora folliculata* resembles that found in other *Heliamphora* species described by Lloyd (1942). Regardless of the shape and specialized structure of *Heliamphora* nectar spoons, the giant nectaries share the uniform architecture in the genus.

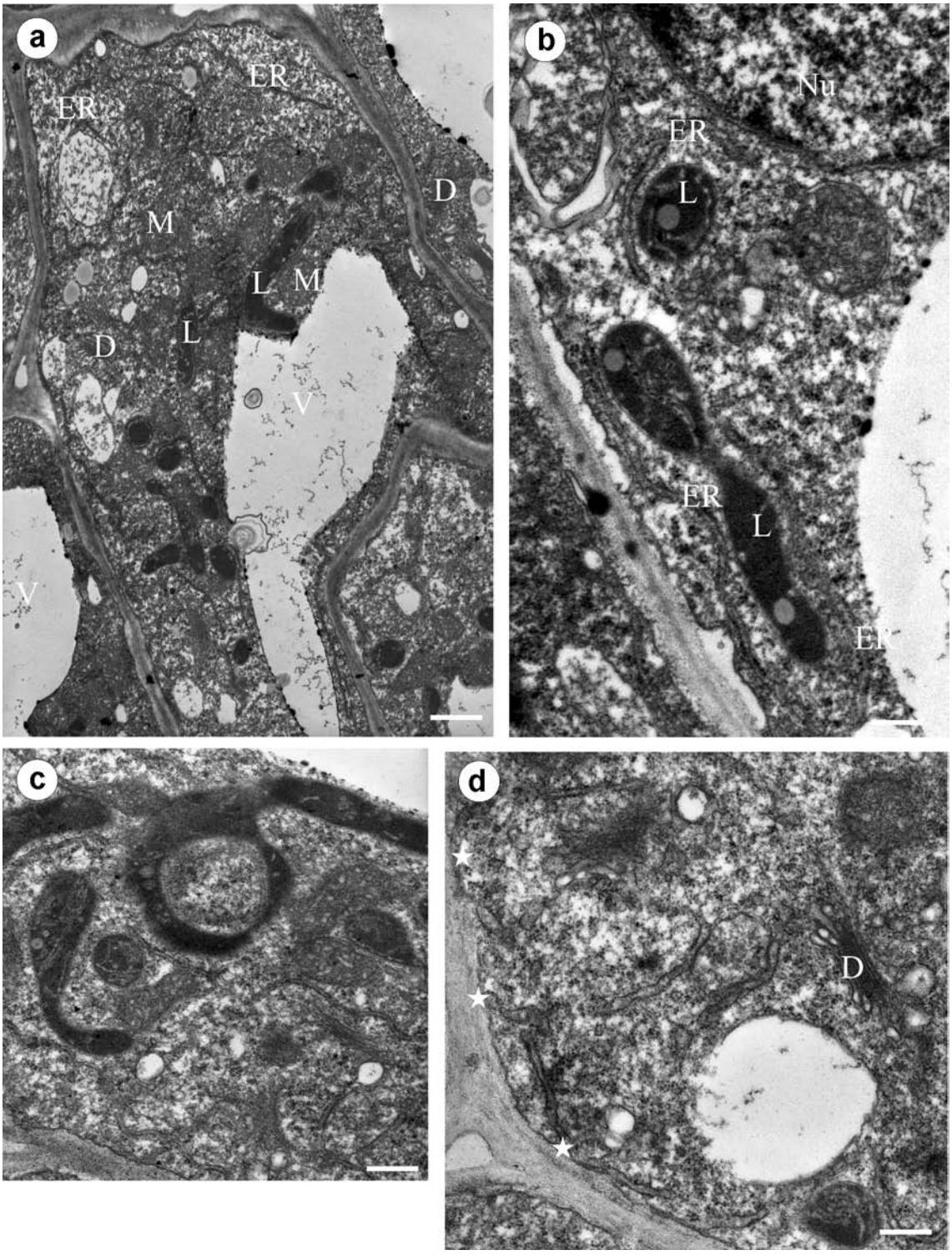

Fig. 5. (**a**) Section through nectary cell: L – leucoplast; V – vacuole; M – mitochondrion; D – dictyosome; ER – endoplasmic reticulum. Bar = 1.4 μm, (**b**) Leucoplasts sheathed by ER in nectary cell. L – leucoplast; Nu – nucleus; rER – endoplasmic reticulum. Bar = 0.35 μm, (**c**) Pleomorphic multilobed leucoplast. Bar = 0.6 μm, (**d**) Part of section through nectary cell, showing cortical ER elements in close contact with plasma membrane (*). D – dictyosome. Bar = 0.5 μm.

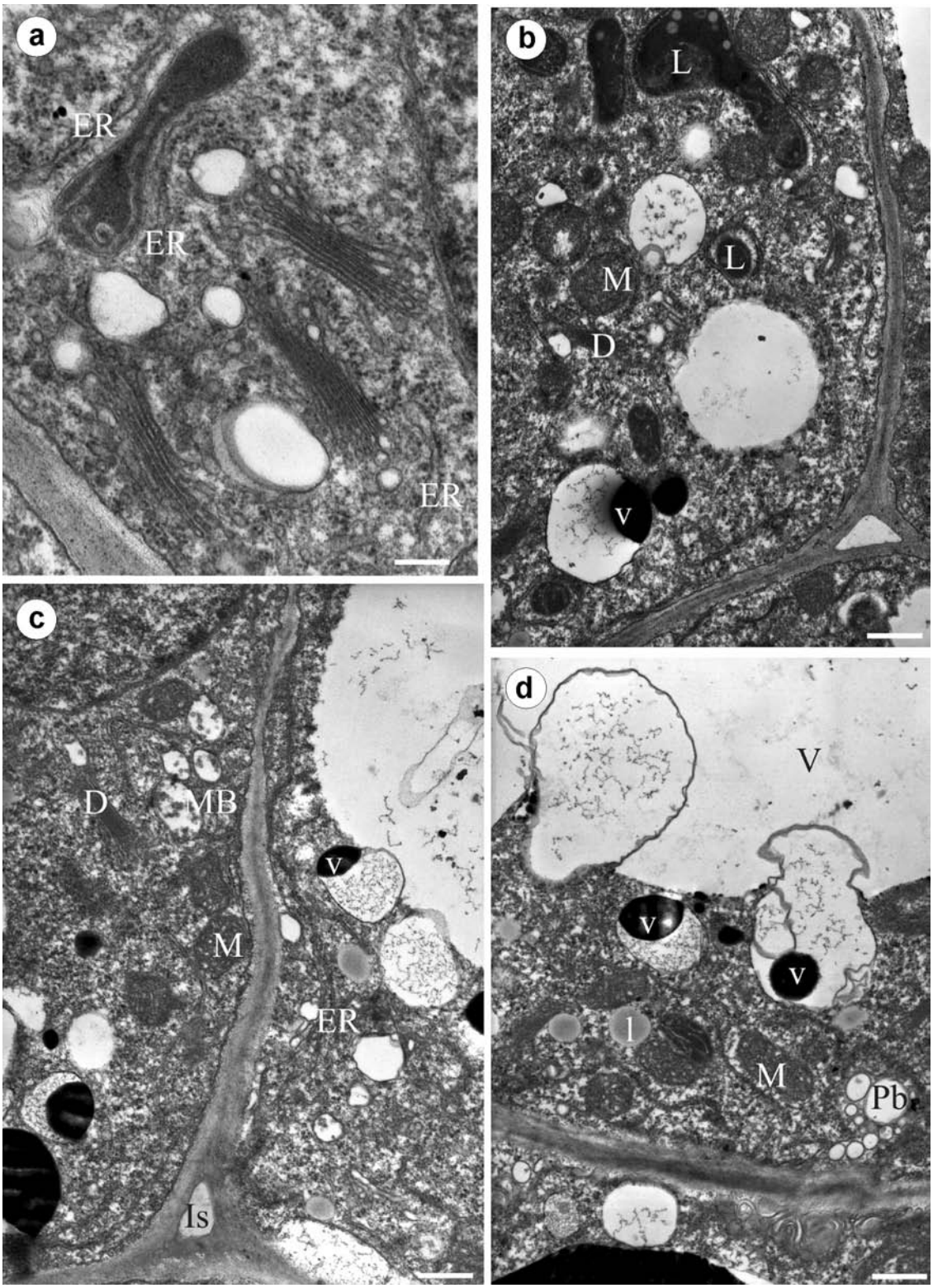

Fig. 6. (**a**) Section through nectary cell. Dictyosomes with large vesicles, ER – endoplasmic reticulum. Bar = 0.3 μm, (**b–d**) Sections through nectary cells, showing vesicles with osmiophilic deposits in cytoplasm, which fuse with the tonoplast to release deposits to the vacuole sap. v – vesicle with osmiophilic deposit; D – dictyosome; Pb – paramural body; ER – endoplasmic reticulum; L – leucoplast; V – vacuole; M - mitochondrion; l – lipid droplet; MB – multivesicular body; Is – intercellular space. Bars = 0.74 μm in (b), 0.55 μm in (c), 062 μm in (d).

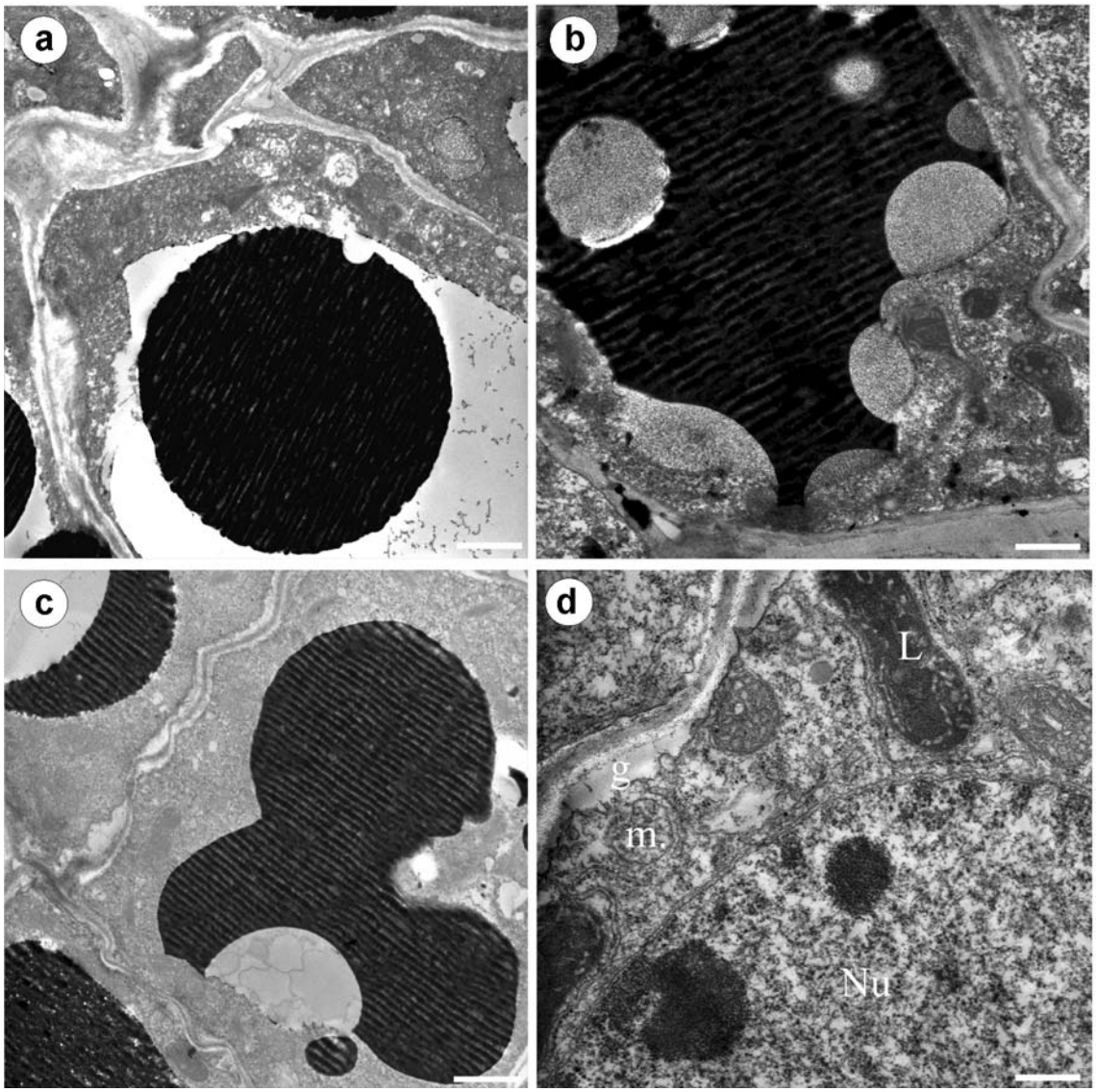

Fig. 7. Section through nectary cells. (**a**) Giant spherical osmiophilic inclusions in vacuole. Bar = 1.5 μm, (**b–c**) Phenolic inclusions filling the whole vacuole; note the appearance of some flocculent material. Bar = 0.75 μm in (b), 1.6 μm in (c), (**d**) Grey material in periplasmic space (g). L – leucoplast; m – microbody; Nu – nucleus. Bar = 0.4 μm.

NECTARIES AS SYMPLASTIC AND APOPLASTIC FIELDS

When a cell or group of cells is symplastically isolated from neighboring cells, it constitutes a symplastic domain; when symplastic transport occurs but is limited, we call it a symplastic field (Rinne and van der Schoot, 1998; Gisel et al., 1999). We find that the *Heliamphora* nectary is a symplastic field. Symplastic transport between the nectary and neighboring parenchyma is possible only between nectary cells and specialized parenchyma cells, which, according to us, are flange cells. Cells with wall thickenings were noted earlier in *Heliamphora* by both Kraft (1896 after Lloyd, 1942) and Lloyd (1942). Lloyd wrote (p. 15): "The function of these parenchyma cells is not known, but it serves to call them transmitting cells. But whether they do more than permit movement of substances from the leaf tissues to the gland, is not known." Kraft maintained that the walls of these cells are cuticularized, but Lloyd thought him mistaken. We found partial cutinization of these walls, confirming Kraft's observation; thus, the *Heliamphora* nectary is an apoplastic field.

Similar cells with wall thickenings have been found in nectaries of the related genus *Sarracenia* (Goebel, 1929–33; Lloyd, 1942). According to Vogel (1998), in *Sarracenia purpurea* these cells have

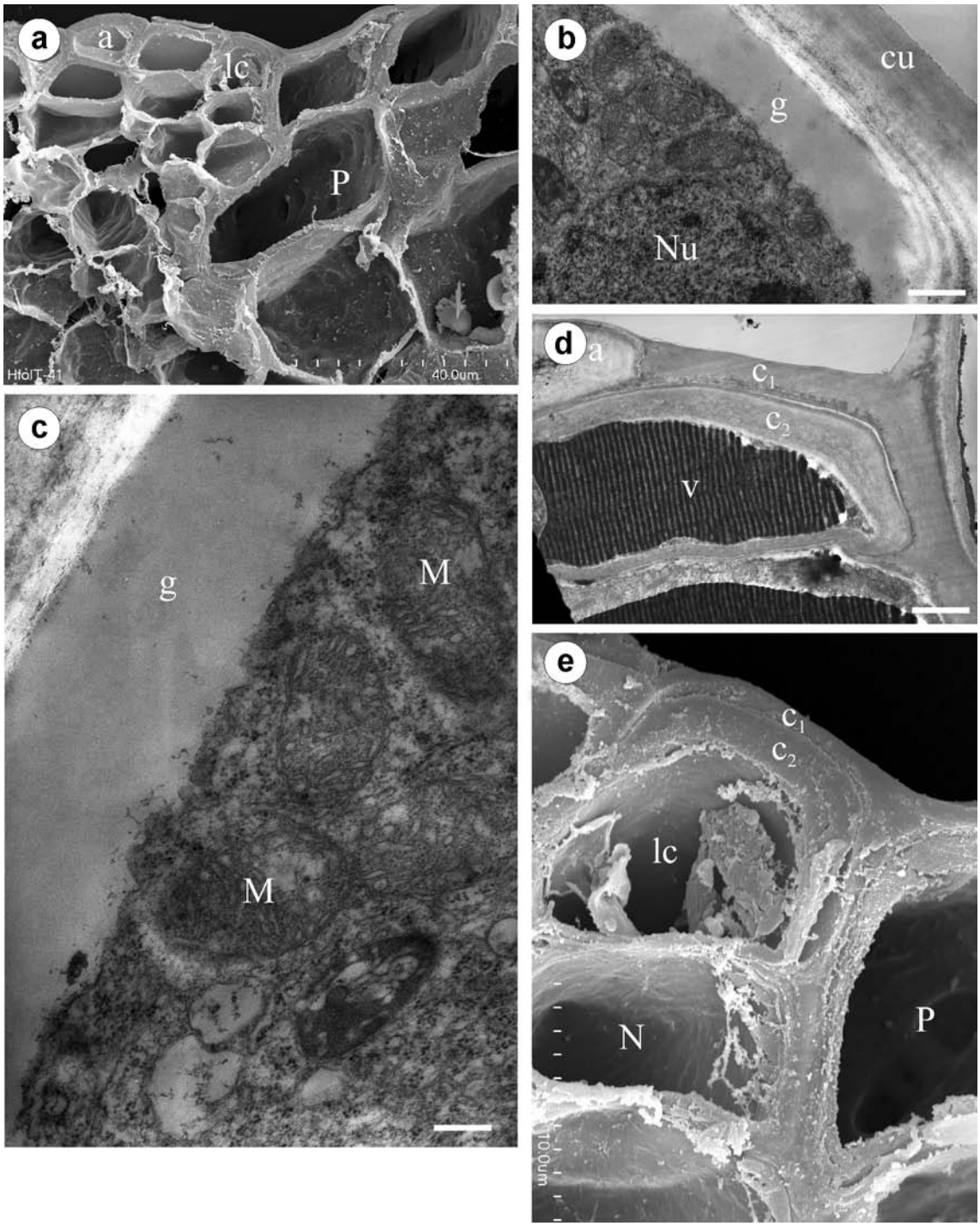

Fig. 8. (**a**) Part of section through giant nectary: a – apical cell; lc – lateral apical cell; P – spoon parenchyma, (**b**) Part of section through apical nectary cell. Under the thin cuticle proper is a thick cuticularized wall. g – amorphous grey material; cu – cuticularized wall; Nu – nucleus. Bar = 0.5 μm, (**c**) Part of section through apical nectary cell; note numerous mitochondria near plasmalemma. g – amorphous grey material; M – mitochondrion. Bar = 0.3 μm, (**d**) Part of section through lateral apical nectary cell; note two-layered outer wall, c_1 – cutinized cell wall layer continuous with cutinized wall of peripheral nectary cells; c_2 – internal cell wall layer; a – apical cell; v – vacuole. Bar = 1.6 μm, (**e**) Part of section through lateral part of nectary, after cytoplasm digestion. lc – lateral apical cell with two-layered outer wall; P – spoon parenchyma; N – nectary parenchyma cell; c_1 and c_2 – two cell wall layer.

walls with lignified ribs and pits, in agreement with our observations in *Heliamphora*. Flange cells occur in sinkers of some parasitic plants of the Viscaceae family; sometimes flange cells are also transfer cells with wall labyrinths, as in *Phoradendron* (Fineran and Calvin, 2000). Thus, both the position (in haustorial or secretory organs) and ultrastructure of these cells show that they are specialized in intensive transport (Fineran and Calvin, 2000; our results).

NECTARY ULTRASTRUCTURE VIEWED IN TERMS OF NECTAR TRANSPORT AND SECRETION

In plants, nectar carbohydrates originate directly from the phloem sap or else as products of photosynthesis in the nectary parenchyma cells (Fahn, 2000; Pacini et al., 2003). In *Heliamphora*, nectar carbohydrates originate from leaf tissues other than nectary cells, which have no chloroplasts. In contrast to the nectaries of several species with plastids containing starch (e.g., Durkee et al., 1981; Durkee, 1982; Nepi et al., 1996; Stpiczyńska et al., 2005), the plastids of *Heliamphora* nectaries are not amyloplasts but ER-sheathed leucoplasts, which is characteristic for secretory cells specialized in monoterpene production (Cheniclet and Carde, 1985). Our conclusions from ultrastructural observations are supported by the analytical work of Jaffé et al. (1995), who found enol diacetal monoterpene (sarracenin), erucamide, phenol, cinerone, phenylacetaldehyde and methyl esters in extracts of the nectar spoon of *Heliamphora*; giant nectaries produce volatile compounds and may have a function similar to osmophores.

The osmiophilic substances in *Heliamphora* vacuoles are very similar to the ergastic substances in vacuoles of the cells in leaf and calyx glands that secrete lipids in *Galphimia brasiliensis* (Malphighiaceae) (Castro et al., 2001). Part of the substances in the vacuoles may represent secreted material. Osmiophilic phenolic substances are common in various secretory tissues; in carnivorous plants they have been reported in nectaries in *Nepenthes* and *Sarracenia*, and in digestive glands of the genera *Drosera, Dionaea, Nepenthes* and *Genlisea* (Akerman, 1917; Barckhaus and Weinert, 1974; Vassilyev, 1977; Juniper et al, 1989; Płachno et al., 2007). The presence of phenolic vacuoles may be correlated with secretion and transport of auxin (Minorsky, 2001).

The occurrence of numerous paramural bodies may suggest a granulocrine mechanism of pre-nectar secretion. Secretory products may also be transported directly from cortical ER to the cell wall via ER membranes connecting with the plasmalemma. The presence of well-developed mitochondria show that pre-nectar transport is dependent on energy. Later, secretory material occurs in the periplasmic space and most probably is transported by the apoplastic route: along cell walls and intercellular spaces. This pathway of transport has been proposed for the nontrichomatous nectaries (e.g., Vassilyev, 1977; Gaffal et al., 1998; Koteyeva et al., 2005; Wist and Davis, 2006). However, in the apical part of the nectary, transport of water-soluble compounds between nectary cells is blocked by cutinized cell walls, unlike lipophilic compounds (e.g., volatile compounds) which may be transported this way. Transport of water-soluble compounds is possible symplastically. Cutinized cell walls, typical of not only nectaries but also other secretory tissue, enable the symplast to control transport (Fahn, 1979; Juniper et al., 1989).

PATHWAY OF NECTAR TO THE NECTARY EXTERIOR

Secretory hairs, glands and nectaries are covered with a cuticle through which secretory products must pass (Fahn, 1979). Secretory products can pass through the cuticle by diffusion when the cuticle is thin or modified, by rupture of the cuticle, through cuticular pores or cuticular gaps as in carnivorous plants, or through modified stomata (Findlay and Mercer, 1971; Fahn, 1979; Juniper et al., 1989; Pacini et al., 2003; Davies et al., 2005). In the absence of cuticular ruptures, pores, or gaps in *Heliamphora*, the release of secretions probably occurs through suitably modified cuticle, but a proper understanding of the pathway of nectar to the nectary exterior in *Heliamphora* nectaries requires further experimental research.

CONCLUDING REMARKS

(1) The special nectary spoon in *Heliamphora folliculata* may have followed an evolutionary sequence via a flattened and later a helmet-shaped spoon. Why has a special spoon with a nectar chamber evolved in *Heliamphora folliculata*? One answer given was that it was to protect against nectar thieving or robbery (for terminology see Inouye, 1980), but this cannot be the case because insects do damage the nectar chamber (Fig. 1b) and nectar robbery does occur (Wistuba, 2001). Exposed nectar readily evaporates (Pacini et al., 2003); the nectar chamber may protect nectar from evaporation, but *Heliamphora* grows in very humid conditions. A better answer may be that it protects nectar against being washed away by frequent rainfall so that the plant produces less nectar and saves energy. Also, when nectar is not easily accessible the insects have to spend more time near the trap entrance to look for it, and they are more likely to be trapped.

(2) In carnivorous plants, when traps and flowers are present at the same time there may be conflict between plant-prey and plant-pollinator systems (Zamora, 1999). According to Jaffe et al. (1992), Formicidae are the most abundant prey in *Heliamphora* traps (possibly excepting *Heliamphora tatei*, which attracts more flying insects). *Heliamphora folliculata* (Wistuba et al., 2001), like other species of this genus (Renner, 1989), is pollinated by bumblebees and produces flowers with pollen as a reward for pollinators (Renner, 1989; Vogel, 1998). So far as is known, pollinator-prey conflict does not exist in *Heliamphora*, and the nectaries in this genus are formed only for nectar-feeding prey. Future detailed studies on the floral nectaries in Sarraceniaceae and related genera should increase our understanding of nectary evolution in New World pitcher plants.

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